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From abiotechnological perspective there are many potential advantages of employing enzymes in organic as opposed to aqueous media. To that end, we have concluded an initial three-year research program in the area of enzymatic catalysis in organic solvents. Our studies have focused on the effect of protein hydration on subtilisins BPN' and Carlsberg in nonaqueous media. Investigations on protein engineered mutants, catalyst engineering studies, and structural studies, primarily employing EPR spectroscopy, have revealed fundamental information on the role of water, the the nature of enzyme structure, and the effects of solvents on the catalytic activity in organic solvents. This study has also resulted in several rational methods to dramatically improve enzyme activity under anhydrous contitions. Catalytic activities in organic solvents can now be expressed at levels similar to that in water--this is a major advance in the field of nonaqueous enzymology.

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BRIEF OUTLINE OF RESEARCH FINDINGS

From a biotechnological perspective there are many potential advantages of employing enzymes in organic as opposed to aqueous media. To that end, we have concluded an initial three-year research program in the area of enzymatic catalysis in organic solvents. Our studies have focused on the effect of protein hydration on subtilisins BPN' and Carlsberg in nonaqueous media. Investigations on protein engineered mutants, catalyst engineering studies, and structural studies, primarily employing EPR spectroscopy, have revealed fundamental information on the role of water, the nature of enzyme structure, and the effects of solvents on the catalytic activity in organic solvents. Our findings can be summarized as follows:

* Protein engineering has been shown to result in catalysts with activities over 100-fold higher than for the wild-type subtilisin BPN' in dry hexane (as a representative organic solvent).

* Dehydration of subtilisin appears to affect the active-site region and destabilize the charged transition-state of the enzyme-catalyzed transesterification reaction. This lost free energy of stabilization may be partially regained by increasing the hydration of the enzyme's active site.

* Addition of 5 μL/mL of water to a THF solution results in a 10-fold increase in the catalytic efficiency of BPN'.

* The local polarity (as measured through EPR spectroscopic studies) increases sharply as water is added up to ca. 20 μL/mL, then levels off at higher water concentrations.

* An independent method to increase the active-site polarity is to modify the active-site structure by site-directed mutagenesis. $Gly_{166} \rightarrow Asn$ (G166N) and $Met_{222} \rightarrow Gln$ (M222Q) mutations were used and showed increased activity relative to the wild-type in THF as compared to water.

* Immobilization of chymotrypsin onto porous glass beads improves catalysis from 17- to 67- fold, depending on the solvent.

* Simple non-buffer salts can <u>dramatically</u> activate enzyme function in organic solvnets. Subtilisin Carlsberg freeze dried into a powder which contained 98% (w/w) KCl, 1% (w/w) enzyme, and 1% (w/w) phosphate buffer was nearly <u>3,750-fold more active</u> in hexane (k_{cat}/K_m = 390 $M^{-1}s^{-1}$) than enzyme prepared in the absence of KCl (whose k_{cat}/K_m is similar to that obtained upon freeze drying the enzyme from 20 mM phosphate buffer).

* A simple approach to the solubilization of enzymes in organic solvents has resulted in catalytic efficiencies similar to that in water. Thus, for subtilisin Carlsberg, values of $k_{cat}/K_m > 4{,}000\ M^{-1}s^{-1}$.

These last two points indicate that enzymatic catalysis in organic solvents can be as efficient as in water. This is critical for the further understanding and use of enzymes in dehydrated environments.

Much of this work is now published or in the process of being published as shown on the following page.

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12. V. Suzawa, Y. L. Khmelnitsky, J. S. Dordick, and D. S. Clark (1994), "Conformational Studies of Partially Hydrated Enzyme Immobilized and Suspended in Organic Solvents", (in preparation)

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Patent Application

J. S. Dordick, D. S. Clark, and Y. L. Khmelnitsky (1994). "Compound and Process to Enhance Activity of Enzymes Suspended in Organic Solvents", U. S. Patent Pending.

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